09:15 h


Recent clinical data have shown that early plasmin proteolysis of human fibrinogen results in the release of fibrinopeptide B (FPB) immunoreactivity into plasma. Upon thrombin treatment this activity increases indicating that the immunoreactivity arises from larger FPB-containing peptides. The presence of degradation products from the BP chain may be an index of fibrinogen proteolysis not only in normal hemostasis but also in pathological disorders such as thrombosis and disseminated intravascular coagulation. Therefore the capability of detecting events leading to progressive plasmin degradation at the BP chain could be of major diagnostic and therapeutic significance. Toward this goal, we have isolated the various peptides released from human fibrinogen by plasmin proteolysis using HPLC on a reversed phase C18 column. These peptides were identified using amino acid analysis and radioimmunoassays for FPB. BP 1-42 was the earliest fragment released during limited plasmin proteolysis. These levels reached a maximum and then began to fall during the course of the digestion. In addition, increasing levels of BP 1-21 and of FPB were shown to lag behind the production of BP 1-42. Using purified BP 1-42 as the target material was shown to occur at the 21-22 bond, with a minor cleavage at the 14-15 bond. Exhaustive digestion yielded two major components, BP 1-42 and BP 22-42 and a minor component composed of BP 15-42. The rate of this reaction is not affected by the addition of hirudin indicating that it is not caused by trace amounts of thrombin. Taken together these data indicate a sequence of events in which BP 1-42 is initially cleaved from fibrinogen during plasmin digestion. Free from its parent molecule, BP 1-42 can undergo further plasmin attack, preferentially at the 21-22 bond to yield BP 1-21 and BP 22-42. Plasmin then attacks BP 1-21 at the 14-15 bond to release FPB.

09:45 h

FIBRINOPEPTIDE A CLEAVAGE AND PLATELET RELEASE IN WHOLE BLOOD IN VITRO. K.L. Kaplan. Department of Medicine, Columbia University College of Physicians & Surgeons, New York, NY, U.S.A.

The relationship between platelet release and fibrinopeptide A (FPA) cleavage from fibrinogen to form fibrin I was examined in blood allowed to clot in vitro. FPA and the platelet release product (thromboglobulin) were quantitated by radioimmunoassay. In undisturbed blood platelet release and FPA cleavage occurred simultaneously. Addition of hirudin inhibited platelet release as well as FPA cleavage, indicating that thrombin was essential for platelet release. Addition of collagen increased platelet release and then FPA cleavage, and addition of ADP had a similar but less marked effect, suggesting that increased platelet activation accelerated thrombin formation. PGE

10:00 h


Thrombin cleaves fibrinogen to release fibrinopeptide A (FPA) and B (FPB) to form fibrin I and II respectively. Initially FPA is cleaved more rapidly than FPB but later the FPA cleavage rate increases as the amount of FPA increases. At the end of an incubation period of 2 hr. FPA studies were made at 1.65 uM fibrinogen concentration, 0.02 U thrombin/ml, pH 7.4, 0.15 M NaCl, 37°. The initial FPA cleavage rate was 7 pmol/min and then increased to 70 pmol/min. The acceleration of FPA cleavage was associated with fibrin polymerization as indicated by light scattering and absorbance measurements. Fibrin polymerization was inhibited by the synthetic tetrapeptide Gly-Pro-Arg-Pro. At a concentration of tetrapeptide of 23 mM (1400 fold molar ratio to fibrinogen) no detectable polymerization occurred over 180 min. At this tetrapeptide concentration the FPA cleavage rate was unaltered from that occurring without tetrapeptide but the rate of FPA cleavage did not show the acceleration normally associated with polymerization although virtually all the FPA was cleaved. The cleavage rates of FPB from fibrin I polymer and monomer were then compared. Fibrin I polymer was prepared by treating fibrinogen with trypsin and fibrin I monomer by similar treatment in the presence of excess tetrapeptide. The cleavage rate from monomer was similar to the initial slow cleavage rate when thrombin was added to fibrinogen. The cleavage rate of human fibrinogen was 25 fold more rapid than that from monomer. These data indicate that thrombin cleaves FPB slowly but completely from fibrin I monomer and at least 25-fold more rapidly from polymer. The data do not establish that thrombin cleaves FPB from fibrinogen.

10:15 h

A NEW, RAPID AND SENSITIVE DETERMINATION OF FIBRINOPEPTIDE-A (FPA) BY A HIGH AFFINITY ANTIBODY TO FPA ANTISERUM. G. Stehle, J. Harenberg, H. Schmidt-Gayk and B. Zimmermann. Department of Clinical Pharmacology and Internal Medicine, University of Heidelberg, FRG

The clinical relevance of the determination of FPA is not yet fully recognized because of the still time consuming radioimmunoassays. Shortening of the incubation times always lead to a critical loss of the sensitivity of the assay. We present now a modification which provides highly sensitive and reproducible results within 2 hrs.

The ethanolic extraction of FPA from plasma was shortened to 20 min including centrifugation. Samples were analysed in triplicates and evaporated at 50°C on microtiter plates. The addition of 0.211 normal rabbit serum (NRS) lead to a 20% increase of the reaction rate of the FPA antisera to FPA. A second antibody with high affinity to rabbit immunoglobulin i.e. the FPA antisera improved the maximal binding to 35% after an incubation period of only 10 min. 35-40000 cps tracer FPA were added to each sample. FPA antisera, second antibody and NRS were preincubated for 1-24 hrs and then added together with the tracer to the samples. Thus a sensitive standard curve was obtained between 0.16 and 160 ng/ml (1200-600 cpm). The correlation coefficient of this modification to our previously described method was 0.9 (n=60). The variation coefficient could be improved substantially to 3.1% for low, 4.0 for medium and 4.4% for high FPA. Normal FPA levels were between 0.16 and 2.5 ng/ml (mean 1.4 ng/ml, n=32).

The present modification of the radioimmunoassay determinate of the difficulties of previously described methods and provides accurate results within 2 hrs.